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# Soil biopore estimation: effects of tillage, nitrogen, and photographic resolution

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**Abstract**

Networks of biopores created by plant and animal activity might accumulate in untilled cropping systems. These would be relatively well connected to the soil surface. The objective of this study was to count biopores after long-term no-till in comparison to recently tilled soil. Biopores were counted and measured to 80 cm depth at 10 cm increments in plots either under no-till wheat production for 1 year or for 17 years, and receiving zero or 130 kg $ha^{-1}$ N. The measurements were repeated using different photographic methods with increased resolution. The only significant difference between the long and short term no-till was in biopore sizes over 1 mm diameter, where long-term no-till produced from 30 to 100% more biopores, probably caused by increased earthworm activity. Over 99% of biopores measured were less than 1 mm diameter. There was no difference between tillage or N treatments in the number of these smaller biopores at any depth. This means small biopores did not accumulate either above or below the plow layer in an untilled cropping system. Improved resolution in the second set of measurements produced a 100-fold increase in detection of biopores in the 0.3–0.5 mm range. This provides evidence that a substantial portion of biopores are very small and were missed in the first year of this study and perhaps in other studies of this type. It is hypothesized that biopores of 0.05–0.5 mm diameter make up over half of total biopore volume and might have a significant role in movement of water and gases. Published by Elsevier Science B.V.

*Keywords:* Biopores; Soil porosity; No-tillage; Wheat

## 1. Introduction

Soil porosity is an important factor in water infiltration. Improved water infiltration is a major motivation in efforts to develop cropping systems which do not rely upon tillage. One physical change which is expected to occur in a soil after a cropping system is changed from one involving tillage to one without tillage is the type and arrangement of soil pores. Many factors such as residue cover, earthworm populations, soil pore continuity, and surface soil structure evolve together when a cropping system is changed to no-till, making it difficult to determine which factors are responsible for increases in infiltration. For example, bare soils with vertical pores may respond very differently than residue covered soils with the same pore structure (Ela et al., 1992). Understanding how tilled and untilled cropping systems differ in pore structure and the infiltration of surface water is important in optimizing cropping systems.

Tillage disrupts biopores generated by biological action of roots, insects, and earthworms and increases the amount of porosity due to random fractures. While total porosity of a tilled soil is often greater than that of an untilled soil (Vermeul et al., 1993), the pores are of

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a different geometry and might have quite different effects on root growth, water infiltration, and gaseous diffusion. For example, Ehlers et al. (1983) found that at depths below 50 cm most roots were growing in biopores. Biopores may also influence the rate of root growth in dry soils (Volkmar, 1996).

Untilled systems would be expected to accumulate biopores in surface layers as well as below tillage depth. Rovira et al. (1987) found that biopore counts were the same when counting biopores >2 mm diameter at a 40 cm depth in tilled versus after 3 years of no-till. Ehlers (1975), however, measured an accumulation of large (>2 mm diameter) biopores caused by earthworms after 4 years of no-till. Since earthworm populations often increase after tillage stops, an increase in large, earthworm-created biopores may reflect the population of earthworms instead of the preservation of biopores where there is no tillage. If biopores accumulate due to a lack of destruction by tillage, then fine biopores left by root growth should be in higher numbers many years after tillage has stopped. This investigation measured biopores in a 17-year no-till experiment in comparison to an adjacent companion experiment which had been under no-till for only 1 year.

## 2. Materials and methods

### 2.1. Experimental site

The experimental plots were part of a long-term no-till experiment established near Pendleton, OR in 1982 on a Walla Walla silt loam (coarse-silty, mixed, mesic Typic Haploxeroll containing about 18% clay, 70% silt and 12% fine to very fine sand). The only soil disturbance in this no-till experiment is due to the 2 cm wide shank on the seed drill which places fertilizer at 10 cm and seed at 8 cm depth. Row spacing is 25 cm. The 2.5 m × 30 m plots were in spring-wheat/winter-wheat (*Triticum aestivum* L.) rotation from 1982 to 1989, when the rotation changed to winter-wheat/fallow. Annual precipitation averages 425 mm, and winter-wheat yields about 5 Mg $ha^{-1}$ with 130 kg $ha^{-1}$ N, and 2.5 Mg $ha^{-1}$ with no fertilizer.

The experimental design is a randomized complete block with 0 and 130 kg $ha^{-1}$ N fertilizer rates in four blocks. Two plots of each fertilizer treatment in each block allow both the fallow and winter-wheat rotation phases to be represented every year. In 1997, the plots were extended lengthwise into a tilled field. In the recently tilled end of each plot, the first no-till winter wheat planting for the rotation entry points was 1997 for one entry and 1998 for the other. Samples were taken in 1998 and 1999 after respective wheat harvest. The sampling areas in the long-term and short-term portions of each plot were 12 m apart.

### 2.2. Biopore measurements

Twenty centimeters diameter intact cores were taken in March 1998 and again in March 1999 from fallow plots using a tractor-mounted soil probe, for a total of 16 cores each year (two nitrogen treatments by two tillage treatments by four replications). Two depth increments, 0–40 and 40–90 cm, were cored using 20 cm diameter plastic tubes. The soil cores were kept field-moist until photographs of horizontal cross-sections were taken.

We used a hydraulic ram to press the cores out of the tubes, stopping when the desired soil depths (5 cm increments to 30 cm depth, 10 cm increments from 30 to 70 cm depth) were at the end of the tube. Pressing the cores out of the tubes did not fracture or compress them. We could then break the core cleanly within 1 cm of our target cross-section depth, which was accurate enough to ensure a clearly focused photograph. Caution was taken to avoid smearing or brushing the exposed surface being prepared because any such physical action obliterated small pores. Compressed air and scissors were used to clean off any dangling roots or protruding soil.

Horizontal cross-sections were photographed using Kodachrome 64 color slide film (Eastman Kodak, Rochester, NY) in 1998. A millimeter scale and sample identification were placed in the corner of each photograph. The slides were then digitized, resulting in a resolution of 12.4 pixels/mm. In 1999, photographs were taken using an Olympus DL600 digital camera (Olympus America, Melville, NY), resulting in a slightly better resolution of 14.9 pixels/mm and, in addition, better edge definition (Fig. 1). The digitized images were cropped to a soil area of 9900 $mm^2$ in 1998 and 1900 $mm^2$ in 1999. Biopores were individually measured and counted using image processing software (Sigmascan, 1998). Biopores

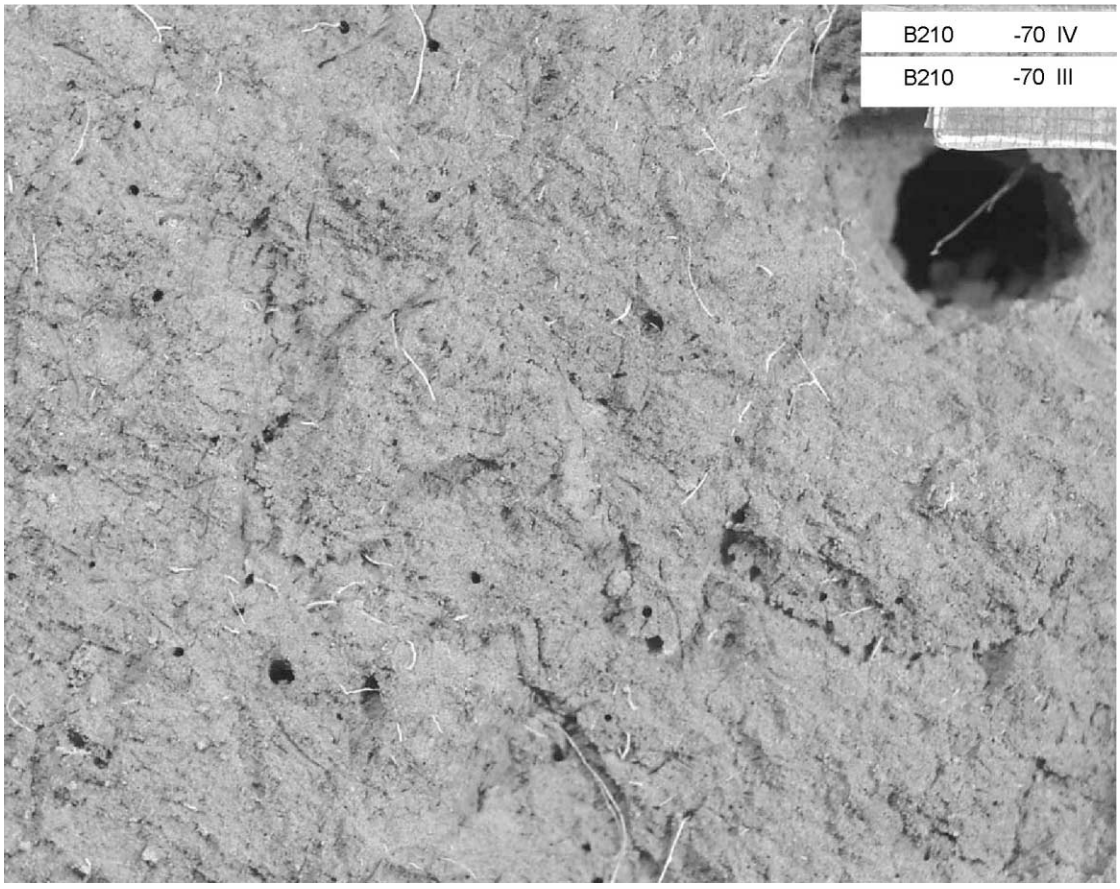


Fig. 1. An example of a digital photograph (1999) at the 70 cm depth (17 years no-till, high N). Graph paper in upper right has 1 mm divisions.

were defined as pores having a smooth, nearly circular perimeter without a visible end in the vertical direction. The digitized images were displayed on a computer screen and biopores identified visually one by one. The perimeter of each biopore was manually traced using the software's elipse-drawing capabilities. The software tabulated the area of the ellipse drawn to match the perimeter of each biopore and calculated the diameter of an equivalent-area, round circle.

Histograms of pore diameter data had the appearance of lognormality, which was confirmed by D'Agostino's test (Parkin and Robinson, 1992). A log transformation would allow the use of statistics designed for normal distributions, but the mean of log transformed data is actually a measure of the median of the original data. In log-normally distributed data, the median can be highly influenced by sample size and not accurately reflect the desired mean. Therefore, uniformly minimum variance unbiased estimators (UMVU) of treatment means were calculated along with Land's method of calculating confidence intervals (Parkin and Robinson, 1992). If the 95% confidence intervals did not overlap for two populations of samples, they were considered significantly different.

## 3. Results and discussion

More biopores were counted in the small diameter categories in 1999 because of increased resolution and edge definition (Fig. 2). Given the measurements taken in 1999, one can see that the drop in frequency of smallest pores in 1998 was not because they are

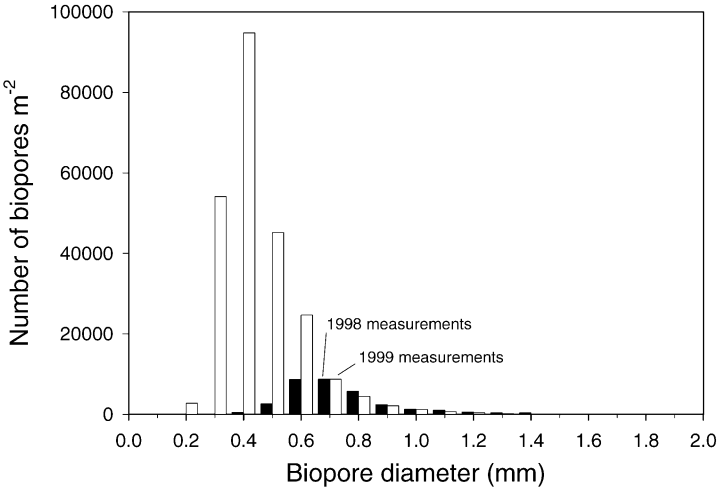


Fig. 2. Frequency distributions of actual pores counted, standardized to $m^{-2}$. Total of individual sample areas counted was 0.85 $m^2$ in 1998 and 0.22 $m^2$ in 1999. Each bar represents a bin spanning 0.1 mm diameter. Improved detection of small biopores is evident in the 1999 measurements.

more rare, but because very few of the small pores were recognizable. After the experiment, inspection of a few soil cores from the same site under 20× magnification in a dissecting microscope with a calibrated reticle revealed that there exist many biopores with diameters of about 0.16–0.08 mm. No biopores less than 0.1 and few less than 0.2 mm diameter were measured using the photographs even in 1999 with improved techniques. This means that very small biopores exist, but could not be detected because they were smaller than the resolving power of our techniques. The shape of the left part of the histograms in Fig. 2 is an artifact of measurement technique and not an indication of true size distribution of the smallest biopores. The increased number of very large pores counted in 1998 relative to 1999 (Table 1) is most

Table 1
Biopore counts expressed on a $m^{-2}$ basis[a]

| Biopore diameter (mm) | 1 year | | | 17 years | | | High N | | | Zero N | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | 95% CI | | Mean | 95% CI | | Mean | 95% CI | | Mean | 95% CI | |
| | | Lower | Upper | | Lower | Upper | | Lower | Upper | | Lower | Upper |
| *Number of biopores ($m^{-2}$), 1998 measurements* | | | | | | | | | | | | |
| 0–0.5[b] | – | – | – | – | – | – | – | – | – | – | – | – |
| 0.5–1 | 23200 | 20600 | 26600 | 24000 | 22600 | 25500 | 24300 | 22500 | 26600 | 22900 | 20700 | 25800 |
| 1–2 | 2500[c] | 2200 | 3000 | 3400[c] | 3000 | 4100 | 3500[c] | 3000 | 4300 | 2500[c] | 2200 | 3000 |
| 2+ | 160[c] | 140 | 180 | 330[c] | 270 | 410 | 290[c] | 240 | 360 | 190[c] | 170 | 230 |
| *Number of biopores ($m^{-2}$), 1999 measurements* | | | | | | | | | | | | |
| 0–0.5 | 188200 | 176300 | 202100 | 207400 | 195800 | 220800 | 195000 | 183500 | 208500 | 200600 | 188200 | 215100 |
| 0.5–1 | 39900 | 34400 | 48000 | 44900 | 38400 | 54700 | 43100 | 37600 | 51000 | 41500 | 35100 | 51300 |
| 1–2 | 1900 | 1300 | 3500 | 1700 | 1300 | 2900 | 2100 | 1400 | 3600 | 1600 | 1200 | 2700 |
| 2+ | 120[c] | 110 | 140 | 190[c] | 160 | 230 | 180[c] | 150 | 220 | 130[c] | 120 | 150 |

[a] The data are shown twice, once comparing 1 year versus 17 years in no-till, and again comparing high versus zero N fertility treatments. Means are of depths and replications, and were estimated using uniformly minimum variance unbiased estimators and confidence interval calculated at the 95% level using the method of Land (Parkin and Robinson, 1992).

[b] 1998 had insufficient resolution in the 0–0.5 mm diameter category.

[c] Mean has a 95% confidence interval that does not overlap that of the comparison treatment (1 versus 17 years in no-till, or high versus zero N).

likely due to the larger sample area. Larger sample area increases the chance of measuring rare pores.

Mercury intrusion methods used on this soil (Pikul and Zuzel, 1994) indicate total porosity in the diameter range 248–0.035 microns is 0.30 $cm^3\ g^{-1}$. This includes both biopores created by plant roots and small organisms plus inter-aggregated spaces. The objective in this study was to measure biopores exclusively, because they constitute a system of connected pathways.

There was no apparent interaction between depth and tillage or N treatment. Estimates of the means and lower and upper 95% confidence intervals reveal that only biopores in the largest size categories (over 1 mm) differ in number between treatments (Table 1). The majority of biopores, which are in the smallest class that these methods could detect, did not differ between 1 year and 17 years of no-till. The differences between mean counts are only 3–13%, so it is not simply large confidence intervals (high variability) which prevents us from considering the biopore counts as significantly different.

The number of large biopores differed significantly when comparing 17 years of no-till versus 1-year of no-till. In 1998, 1–2 mm biopores had 36% greater count per area and 2+ mm biopores had 106% greater count per area after 17 years of no-till. The 1999 measurements only show the 2+ mm biopore counts

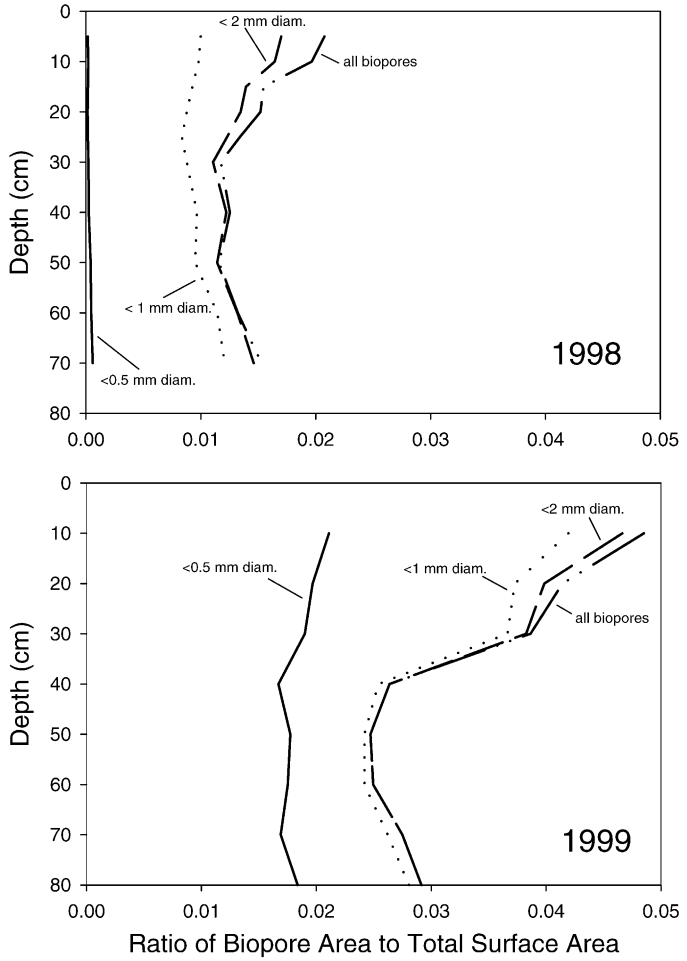


Fig. 3. Relationship of biopore areas and depth expressed as a proportion of soil surface area. An improvement in our ability to detect small biopores in 1999 resulted in a large increase in the estimate of total biopore area.

to have non-overlapping confidence intervals, again with the longer term no-till having more large biopores.

When the same data are grouped into zero and high N rate treatments, a greater number of large pores in the 1–2 and 2+ mm categories (1998), and the 2+ mm category (1999) occurred in the high N-rate treatment.

This data does not support the hypothesis that soil untilled for 17 years accumulates biopores compared to soil planted for the first time without tillage. The increase in larger pores can be attributed to the increase in earthworm population measured in the 17 years no-till plots (data not shown). The earthworm population may have been influenced by nitrogen treatments through the increase in biomass production and surface cover at high N rates.

It can be assumed that each wheat crop produces a substantial number of new biopores. These biopores apparently have a short life in this Walla Walla silt loam, even if not disturbed by tillage. An alternate explanation might be that roots predominately grow into existing biopores. The lack of accumulation of biopores below the tillage layer has been reported in other studies comparing tilled and untilled cropping systems (Gantzer and Blake, 1978; Rovira et al., 1987).

The pore area created by the smallest biopores is constant with depth (Fig. 3). The biopore area contributed by 0.5–1 mm diameter pores increases near the surface, where root mass is greatest. Biopores over 1 mm occur almost exclusively above 30 cm depth. One should keep in mind that our technique missed pores less than a certain diameter. The 1998 measurements missed a majority of the soil's total biopore area, as shown by 1999 data. This points to the possibility that even smaller biopores represent a significant, if not a majority, proportion of actual biopore area. Likewise, Edwards et al. (1988) found that the smallest biopores measured (0.4 mm diameter) were in the greatest abundance and produced the majority of the biopore area. Wilson and Luxmoore (1988) found a two order-of-magnitude increase in the number of pores as the size class decreased from 1.5 to 0.2 mm, based on calculations from hydraulic conductivity measurements.

Unless a particular research objective provides a rationale for ignoring biopores smaller than a particular size, a limitation in identification of small biopores will limit the ability to draw conclusions. It is possible that biopores beyond the resolving power of this experiment, i.e., smaller than about 0.3 mm diameter, make up a significant proportion of total biopore volume. Most biopores are created by roots and form continuous channels from the soil surface to the entire soil volume exploited by the plant. Even the smallest biopores could be significant in the movement of liquids, gasses, or organisms.

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